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The distribution and calls of Vraem' Treefrog, *Dendropsophus vraemi* (Caminer, Milá, Jansen, Fouquet, Venegas, Chávez, Lougheed, and Ron 2017), with comments on its conservation status

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Abstract.—Dendropsophus vraemi **is a recently described frog that is only known from the type locality, in La Mar province, Ayacucho, Peru. Here, we present new data on its geographic distribution, coloration in life, and habitat, as well as descriptions of the advertisement and aggressive calls of this species. The new localities extend the known distribution range 151 km northwest and the elevation range down to 250 m asl, which is nearly 450 m lower than previously known. Our findings are inconsistent with its current IUCN categorization of Least Concern (LC), and we suggest that this species should be categorized as Data Deficient.**

Keywords. Amphibia, Anura, Data Deficient, Peru, South America, vocalization

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Introduction

Phylogenetic analyses have given scientists a new approach for solving taxonomic riddles which had remained unresolved for years. Nevertheless, a genetic approach should not be the only basis for assigning new taxonomic positions, but it can serve as an important tool that helps in performing a complete and accurate analysis. Traditional features are still important to support the establishment of species limits and to identify species in the field and laboratory. Researchers tend to identify amphibians using mostly morphological and acoustic characters, and these characteristics continue to provide strong support for analyses of proper species diagnosis.

Dendropsophus vraemi is a recently described frog that has been placed in the *Dendropsophus leucophyllatus-triangulum* complex (Caminer et al. 2017) and the *D. leucophyllatus* group by Orrico et al. (2020). Thus far, it is only known from the type locality. This species is genetically and morphologically distinct from any other known *Dendropsophus*. It has a unique combination of characters consisting of a pale triangular mark on the tip of the head with its base in the interorbital area and its apex between the nostrils, two to four marks on the sacrum, a particular shape of marks on the dorsum (thin on the sides), a yellowish venter, and orange or pink coloration on the digits, webbing, axillary membranes, groin and hidden surfaces of the arms and legs (Caminer et al. 2017). The advertisement call of this frog is unknown, and here we use data collected from additional field surveys to describe the call and add new locality data which extend the distribution of this poorly-known species.

Materials and Methods

Study area. The fieldwork was performed in two localities: one in the middle Apurimac basin near the type locality, and one near a small tributary of the lower Apurimac basin (Fig. 1). Both are rural areas adjacent to the Andean foothills of the Apurimac river basin. The habitat on both sites consisted of temporary ponds, about 100 m from the river, surrounded by grassy vegetation and bushes about 2 m tall. No trees or plants providing canopy shade were recorded. Each pond was approximately 400 m^2 and no more than 10 cm in depth. There were cacao, coffee, and palm farms adjacent to the ponds.

Field sampling. We carried out fieldwork during the dry season of September 2016 in the middle Apurimac basin, and in May 2019 in the lower basin. We used Visual

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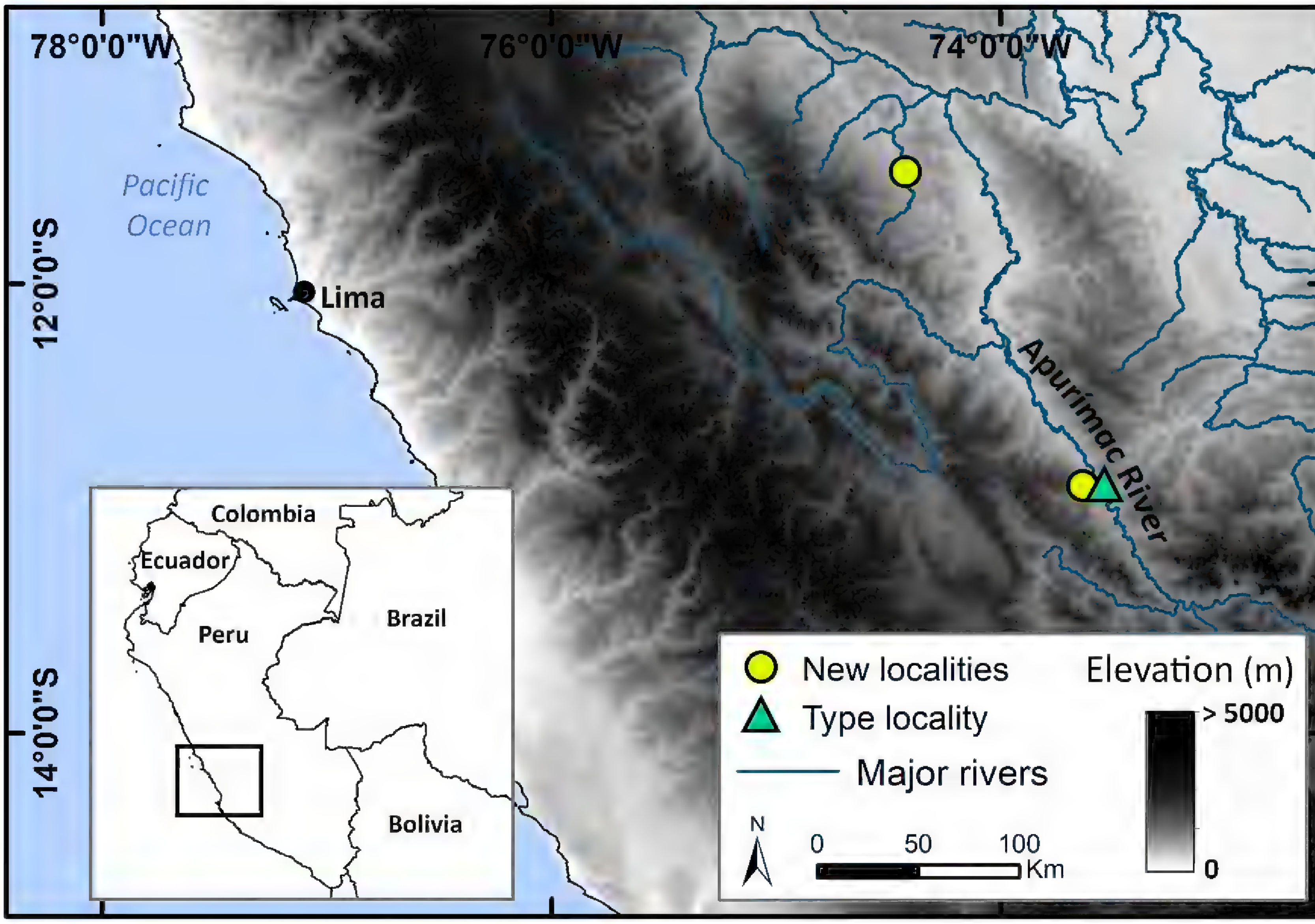

Fig. 1. Geographic distribution map for *Dendropsophus vraemi* showing the type locality (green triangle; Caminer et al. 2017) and new localities in the middle and lower Apurimac basin (yellow circles), extending the range by 151 km to the northwest.

Encounter Surveys (Crump and Scott 1994) to survey amphibians at night. Photographs taken in the field were used for descriptions of coloration in life. The specimens collected from the two localities were fixed in 10% formalin and then stored in 70% ethanol under the permit RD-414-2016-SERFOR/DGGSPFFS. We recorded calls with a Marantz PMD-222 professional recorder coupled to a Sennheiser MS 907 unidirectional microphone (13 September 2016; air temperature 27 °C). Recordings and voucher specimens are deposited at Division de Herpetología - CORBIDI, Lima, Perú.

Call analysis. Recordings were digitized at a resolution of 16 bits, with a 44,100 hz sampling rate. To allow for direct comparisons with other related species, we followed the call parameters used by Caminer et al. (2017): call duration(s), number of notes per call, rise time of the call(s), number of pulses per call, note duration(s), number of pulses per note, distance between notes, average of the dominant frequency call (Hz), and frequency bandwidth (Hz). For the purpose of analyzing the call of *D. vraemi,* we defined frequency bandwidth as 90% bandwidth. We analyzed calls with a Fast Fourier Transformation window of 1,024 points and the Hann algorithm, all other parameters used default settings in Raven Pro 1.5 (Cornell Lab of Ornithology, Ithaca, New York, USA). Oscillogram and spectrogram figures were produced using the packages seewave (Sueur et al. 2008), tuneR (Ligges et al. 2018), and ggplot2 (Wickham 2016) in R version 3.5.1 (R Core Team, 2018) with a Fast Fourier Transformation window length of 1,024, and overlap of 90. We followed Toledo et al. (2015) to classify the advertisement and aggressive calls, Köhler et al. (2016) and Caminer et al. (2017) to describe call characteristics, and Köhler (2012) for the description of the color pattern.

Results

On 13 September 2016, Germán Chávez (GC) and Andy C. Barboza (ACB) caught three male individuals (CORBIDI 17893–95, Fig. 2), in the vicinity of San Antonio, La Mar province, Ayacucho, Peru (12°55'46.8"S, 73°32'3.3"W; 693 m asl), middle basin of Apurimac River (hereafter referred to as the middle Apurimac basin) and only 5 km from the type locality. On 4 May 2018, GC caught two more males (CORBIDI 21857–58, Fig. 2E–H), in Boca Kiatari Native Community, Satipo province, Junin department, Peru (11°31'38.9"S, 74°24'40.2"W; 210 m asl), lower basin of Apurimac river (hereafter referred to as the lower Apurimac basin). The characteristics of all individuals match the morphological features described by Caminer et al. 2017 (see Fig. 2), however we noted some variations in the measurements and dorsal coloration which are detailed in Table 1 and also described below. These new records add two localities and extend the distribution range of *D. vraemi* by 151 km to the northwest (Fig. 1).

At both localities, the captured individuals were calling from the water at night between 1900 and 2000 h (the water level did not pass over their heads) in ephemeral flooded areas (approximately 400 m^2), covered by

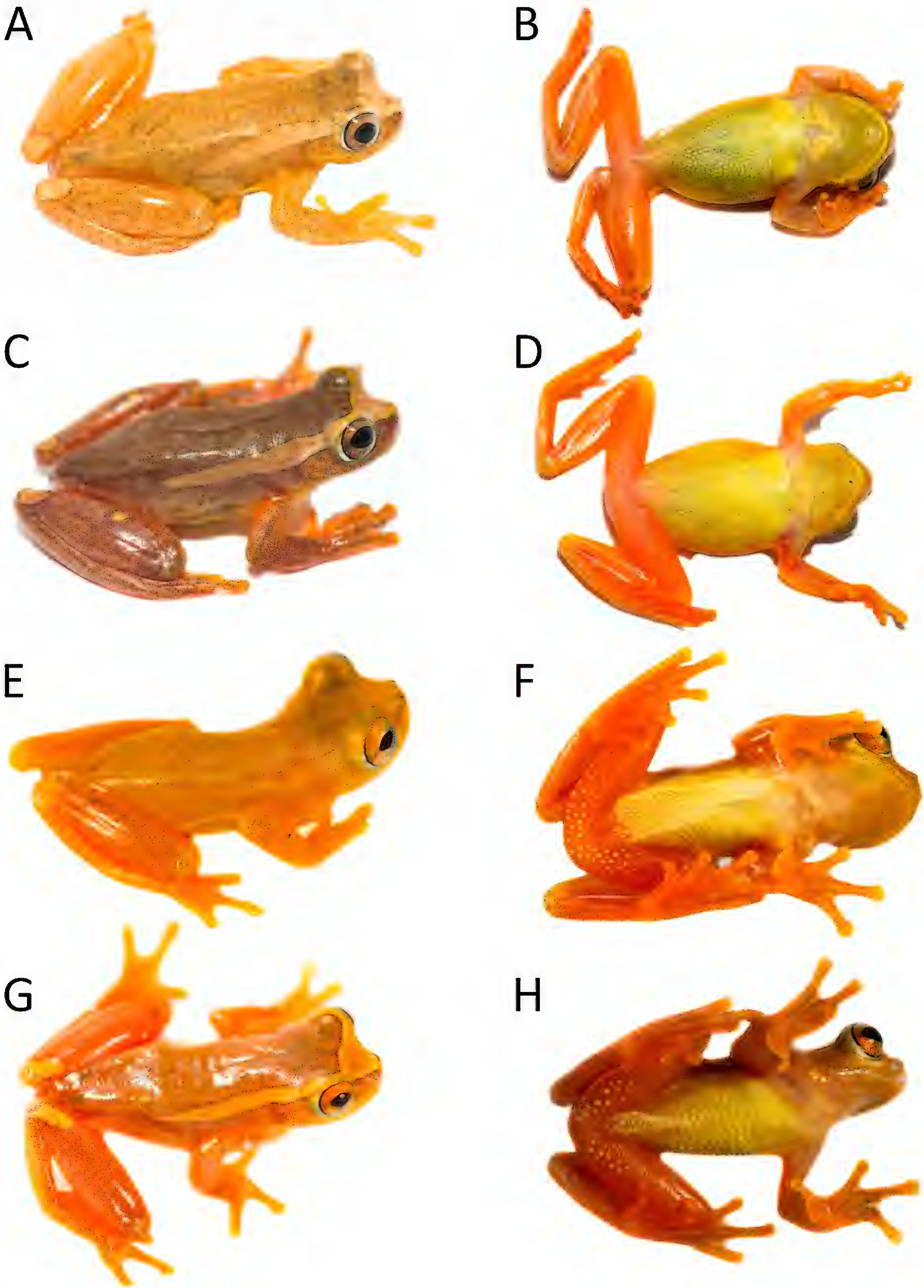

Fig. 2. Dorsolateral and ventral views of *Dendropsophus vraemi* in life. **A–B:** CORBIDI 17894 (SVL = 26.4 mm); **C–D:** CORBIDI 17895 (SVL = 26.1 mm); **E–F**: CORBIDI 21857 (SVL = 22.8 mm); **G–H:** CORBIDI 21858 (SVL = 22.9 mm).

Table 1. Descriptive statistics of the morphological measurements (in mm) of *Dendropsophus vraemi* from two new localities in the lower (CORBIDI 21857–58) and middle (CORBIDI 17893–95) Apurimac River basin. Descriptive statistics indicate mean ± standard deviation and range.

	San Antonio, middle Apurimac basin (*n* = 3)	Boca Kiatari, lower Apurimac basin (*n* = 2)
SVL	26.0 ± 0.5 (25.4–26.1)	22.8–22.9
HL	7.3 ± 0.1 (7.2–7.5)	7.2–7.3
HW	7.9 ± 0.1 (7.8–8.1)	7.0–7.3
TL	13.0 ± 0.2 (12.7–13.1)	11.6–11.6
FL	11.8 ± 0.8 (10.9–12.5)	9.9–10.2
Femur length	12.4 ± 0.1 (12.2–12.5)	10.5–10.8
Hand length	7.6 ± 0.3 (7.3–8.0)	7.2–7.4
HL/SVL	0.2 ± 0.0 (0.2–0.2)	0.3–0.3
HL/HW	0.9 ± 0.0 (0.9–0.9)	0.9–1.0
HW/SVL	0.3 ± 0.0 (0.3–0.3)	0.3–0.3
TL/SVL	0.5 ± 0.0 (0.4–0.5)	0.5–0.5
FL/SVL	0.4 ± 0.0 (0.4–0.4)	0.4–0.4

herbaceous vegetation and small bushes. In both sites, our specimens were part of a larger chorus of males (~15–20 males, GC field notes). The distance between males was approximately 50–80 cm, and the only frog species recorded in sympatry with *D. vraemi* were in the genus *Leptodactylus*.

We recorded two types of calls, all from one male in the middle Apurimac basin (CORBIDI 17894): 1) the advertisement call, in two different variations (Fig. 3 A–B), and 2) the aggressive call (Fig. 3C). The first variation of the advertisement call consists of two pulsed notes with a call duration of 0.70 s, 106 pulses per call, a call rise time of 0.09 s, an average dominant frequency of the call of 2,258.37 Hz, and a frequency bandwidth of 750.00 Hz, without a secondary note type. The second variation of the advertisement call has a duration of 1.24 s, consists of three pulsed notes with an average dominant frequency of call of 2,431.51 Hz, a call rise time of 1.05 s, and frequency bandwidth of 937.50 Hz. The aggressive call has a duration of 1.53 s, consists of three pulsed notes with an average dominant frequency of call of 2,269.44 Hz, a call rise time of 0.39 s, and frequency bandwidth of 750.00 Hz, without a secondary note type. For details of the call variables and comparisons with other species of the *Dendropsophus leucophyllatus-triangulum* complex (*sensu* Caminer et al. 2017), see Tables 2 and 3.

We also found some phenotypic differences between the two specimens collected from the lower Apurimac basin (CORBIDI 21857–58, Boca Kiatari Native Community, Junin department), and the three specimens collected from the middle Apurimac basin (CORBIDI, 17893–95). First, specimens from the lower Apurimac basin have a smaller SVL than specimens from the middle Apurimac basin (SVL range lower Apurimac = 22.8–22.9 mm; middle Apurimac = 25.4–26.1 mm; Table 1). Second,

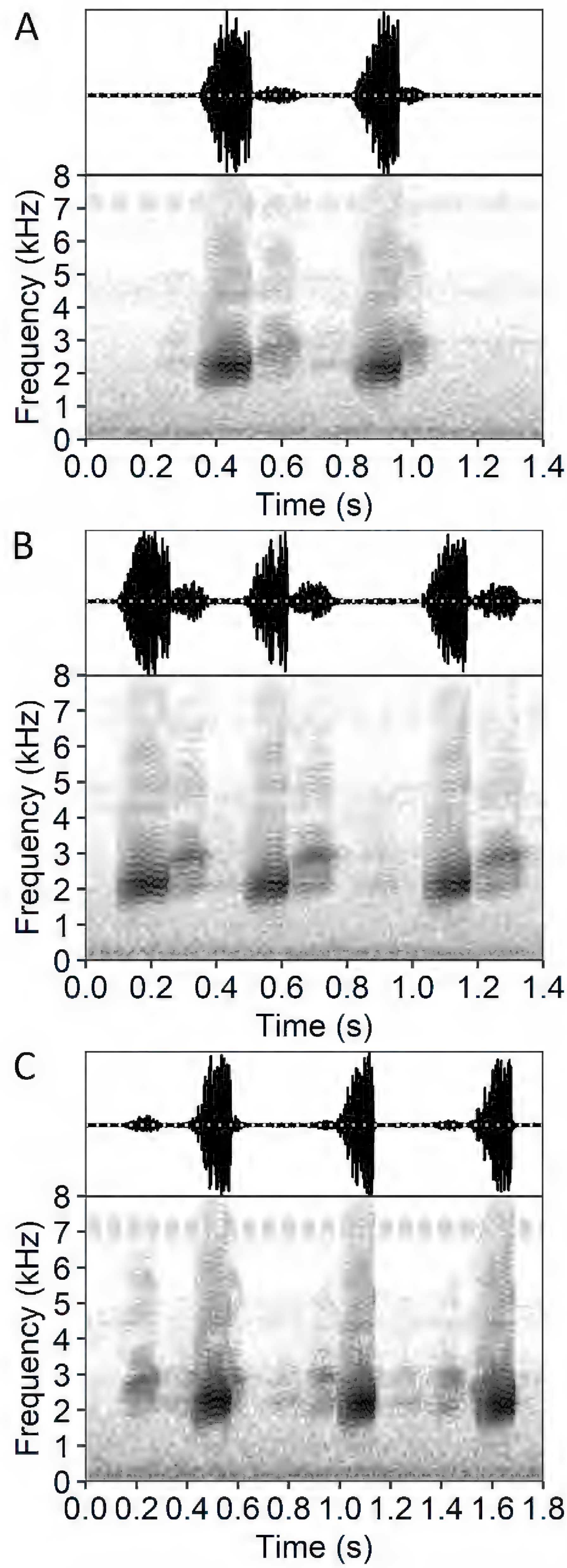

Fig. 3. Spectrograms and oscillograms for *Dendropsophus vraemi*. **A–B:** variations of the advertisement call; **C**: an aggressive call. **A, B** and **C** are call variations from a single male individual (CORBIDI 17894) recorded in the middle Apurimac basin.

Table 2. Descriptive statistics of the advertisement call of *Dendropsophus vraemi* (this study) and species in the *D. leucophyllatus-triangulum* complex (Caminer et al. 2017). Descriptive statistics are indicated as mean ± 1 SD and range in parentheses.

	Call duration(s)	Number of notes per call	Rise time of the call(s)	Number of pulses per call	Type I note duration(s)	Number of pulses of the type I note	Type II note duration	Number of pulses of the type II note	Distance between notes(s)	Average of the dominant frequency call (Hz)	Frequency bandwidth (Hz)
Dendropsophus vraemi (*n* = 2)	0.97 (0.70–1.24)	2.50 (2–3)	0.57 (0.09–1.05)	136.50 (106–167)	0.28 ± 0.03 (0.23–0.31)	54.60 ± 7.64 (43–63)	No data	No data	0.18 ± 0.08 (0.11–0.26)	2,344.94 (2,258.37–2,431.51)	843.75 (750.00–937.50)
Dendropsophus leucophyllatus (*n* = 9)	0.17 ± 0.02 (0.16–0.22)	2.10 ± 0.16 (2–3)	0.08 ± 0.04 (0.05–0.15)	16.49 ± 1.94 (14–21)	0.1 ± 0.01 (0.08–0.11)	12.19 ± 0.91 (10.8–13.8)	0.027 ± 0.007 (0.01–0.004)	3.85 ± 0.45 (3.60–4.25)	0.046 ± 0.006 (0.03–0.05)	2,748.69 ± 162.89 (2,453.9–2,914.1)	530.32 ± 113.7 (301.4–663.2)
Dendropsophus arndti (*n* = 7)	0.19 ± 0.02 (0.16–0.23)	1	0.12 ± 0.08 (0.03–0.29)	17.44 ± 1.23 (16–19)	0.2 ± 0.02 (0.16–0.23)	17.44 ± 1.23 (15.6–19.0)	No data	No data	No data	2,655.37 ± 169.42 (2,416.3–2,876.1)	487.67 ± 75.06 (353.1–570.6)
Dendropsophus reticulatus (*n* = 4)	0.35 ± 0.05 (0.27–0.39)	4.05 ± 0.33 (3–5)	0.18 ± 0.01 (0.18–0.19)	30.39 ± 2.75 (26–33)	0.1 ± 0.01 (0.08–0.12)	13.55 ± 1.18 (12.6–17.0)	0.039 ± 0.004 (0.03–0.04)	5.56 ± 0.47 (5–6)	0.042 ± 0.004 (0.03–0.04)	2,992.39 ± 100.80 (2,888.3–3,128.6)	705.98 ± 112.5 (574.2–843.7)
Dendropsophus triangulum (*n* = 11)	0.26 ± 0.04 (0.22–0.36)	2.33 ± 0.52 (2–4)	0.13 ± 0.03 (0.09–0.17)	20.92 ± 3.39 (17–29)	0.15 ± 0.02 (0.12–0.18)	15.72 ± 2.24 (13.0–20.2)	0.034 ± 0.007 (0.023–0.042)	3.75 ± 0.52 (3.0–4.5)	0.051 ± 0.007 (0.04–0.06)	2,456.43 ± 278.91 (2,101.0–3,070.7)	478.34 ± 113.54 (281.2–646.0)

the lower Apurimac specimens have a yellow background color pattern in life, which is an addition to the range of background colors described by Caminer et al. (2017). The male CORBIDI 17894 from the middle Apurimac basin has a creamy yellow dorsal background, but not properly yellow. Third, the dorsolateral bands in lower Apurimac basin specimen CORBIDI 21857 are weakly differentiated from the dorsum background, having mostly a yellowish background inside the band. Last, the iris in both lower Apurimac individuals is bronze-yellow and not gray bronze as in the type series and in our specimens from the middle Apurimac basin.

Discussion

The specimens reported here confirm that this species occurs in the Andean foothills to the west of the Apurimac River by an extension of at least 151 km northeast of the type locality. The presence of *D. vraemi* on the eastern side of the river remains uncertain. However, we believe that the lack of records to the east of the river likely reflects the scarcity of fieldwork performed in those areas instead of the possible role of the Apurimac as a riverine barrier. The Apurimac river causes geographic separation in some birds in its upper basin (Hosner et al. 2015), where canyons and dry forests create a rough topography. Otherwise, this phenomenon is uncommon in the lower areas where tropical rainforest and a flat relief is the dominant habitat on both sides of the river. Similar ecosystems have already been reported as semi-permeable barriers in Amazon lowlands (Moraes et al. 2016). Therefore, we suggest that further field surveys are necessary to confirm the presence of *D. vraemi* on the eastern side of Apurimac River. The new records also indicate that this species inhabits areas as low as 250 m asl, nearly 450 m lower than previously known (Caminer et al. 2017). Furthermore, the ecosystem is the same as that of the type series: the eastern Andean foothills.

Compared to the other species in the *D. leucophyllatus-triangulum* complex, the mean duration of the advertisement calls of *D. vraemi* is 0.80–0.62 s longer than the mean duration of the advertisement calls of the other species. The one aggressive call of *D. vraemi* analyzed is 1.39–0.94 s longer than the mean duration of aggressive calls of other species (Table 2). The longer duration is a consequence of both longer note durations and longer distances between notes. The average of the dominant frequency of the advertisement call of *D. vraemi* tends to be lower than those of other species described by Caminer et al. (2017), and only overlaps the lower range of *D. arndti* and *D. triangulum* for this call characteristic. Likewise, the average of the dominant frequency of the aggressive call appears to fall within the lower bounds of the range for the species (Tables 2 and 3).

Our measurements indicate that middle Apurimac basin specimens fall within the type series SVL range (25.1–27.6 mm), whereas the specimens from lower

Table 3. Descriptive statistics of the aggressive calls of *Dendropsophus vraemi* (this study) and species in the *D. leucophyllatus-triangulum* complex (Caminer *et al.* 2017). Descriptive statistics indicate mean ± 1 SD and range in parentheses.

	Call duration(s)	Number of notes per call	Rise time of the call(s)	Number of pulses per call	Note duration(s)	Number of pulses per note	Distance between notes(s)	Average of the dominant frequency call (Hz)	Frequency bandwidth (Hz)
Dendropsophus vraemi (*n* = 1)	1.53	3	0.39	167	0.35 ± 0.100 (0.26–0.47)	55.67 ± 8.50 (47–64)	0.25 (0.24–0.26)	2,269.44	750.00
Dendropsophus leucophyllatus (*n* = 5)	0.25 ± 0.05 (0.18–0.34)	3.96 ± 0.64 (3–5)	0.15 ± 0.15 (0.04–0.4)	19.96 ± 4.14 (13–25)	0.033 ± 0.004 (0.027–0.038)	5.01 ± 0.36 (4.6–5.5)	0.036 ± 0.004 (0.029–0.040)	2,615.82 ± 196.39 (2,326.6–2,811.9)	548.96 ± 101.28 (469.4–689.1)
Dendropsophus arndti (*n* = 7)	0.14 ± 0.01 (0.12–0.16)	3.03 ± 0.08 (3–4)	0.07 ± 0.02 (0.03–0.1)	11.60 ± 1.13 (12–13)	0.035 ± 0.006 (0.024–0.042)	3.84 ± 0.40 (3.13–4.33)	0.02 ± 0.011 (0.011–0.045)	2,668.76 ± 111.64 (2,485.2–2,830.3)	423.26 ± 64.73 (335.9–516.8)
Dendropsophus reticulatus (*n* = 5)	0.59 ± 0.11 (0.48–0.71)	4.11 ± 0.72 (3–5)	0.38 ± 0.13 (0.24–0.51)	22.11 ± 5.71 (16–29)	0.038 ± 0.003 (0.035–0.041)	5.32 ± 0.53 (4.5–5.8)	0.005 ± 0.004 (0.045–0.055)	2,832.05 ± 106.24 (2,675.2–2,924.1)	735.46 ± 120.51 (600.0–875.0)
Dendropsophus triangulum (*n* = 5)	0.24 ± 0.03 (0.19–0.27)	4.25 ± 0.23 (4–5)	0.14 ± 0.02 (0.11–0.16)	19.81 ± 1.66 (17–22)	0.036 ± 0.005 (0.034–0.041)	4.66 ± 0.35 (4.3–5.1)	0.027 ± 0.006 (0.018–0.033)	2,411.74 ± 349.62 (2,085.0–2,968.3)	427.4 ± 187.34 (281.2–689.1)

Apurimac are smaller (see Table 1). Furthermore, the color pattern variation that we found slightly increases the range of background colors described for the species (from brown or gray to yellow). Therefore, we suggest that the coloration patterns vary geographically. Unfortunately, females have not been found thus far, and consequently their coloration remains unknown.

According to Caminer et al. (2017), males of the type series of *D. vraemi* were perched on leaves of the vegetation alongside a stream. However, we spotted them on the ground in small temporary ponds partially covered by water. It is important to mention that the ponds in our surveys lacked trees and only herbs or small bushes were present, which suggests that this species can vary its perching places depending on the habitat it occupies. Our observations suggest that this species can inhabit disturbed areas that lack canopies. Despite its tolerance to disturbance, our findings are inconsistent with the current IUCN Least Concern (LC) categorization. The small known area of occurrence (only known from three localities no more than 150 km apart) and lack of information on abundance leads us to recommend that this species should be placed into the Data Deficient category of the IUCN Red List, as previously recommended by Caminer et al. (2017).

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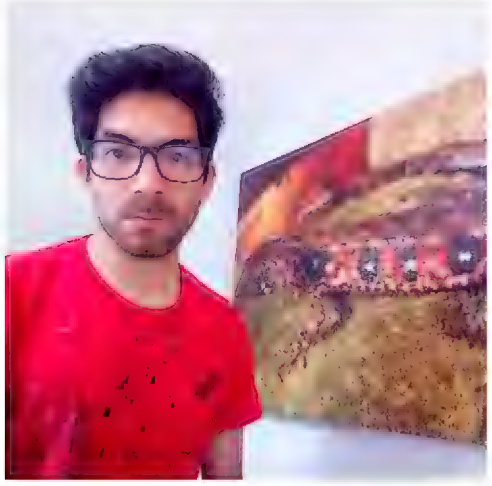

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Andy C. Barboza is a Peruvian scientist who graduated in Biological Sciences from Universidad Nacional de Trujillo, La Libertad, Peru, in 2012. She currently collaborates with the Herpetology Collection of the Centro de Ornitología y Biodiversidad (CORBIDI), increasing her deep interest in amphibians. For her undergraduate thesis, she worked on the composition and altitudinal distribution of amphibians from Otishi National Park, in collaboration with the Missouri Botanical Garden (GMB). Her current research interests focus on the systematics, diversity, and conservation of Neotropical herpetofauna, particularly in Peru, as well as the evolutionary responses and behavior of amphibians due to climate change.

Michelle E. Thompson is a Conservation Ecologist/Herpetologist on the Rapid Inventory team at the Field Museum of Natural History in Chicago, Illinois, USA. She is interested in the application of population and community ecology to species conservation and understanding the patterns of diversity and distributions of tropical amphibians and reptiles.